Nicotine-Treated *Fusobacterium nucleatum* Binding to Collagen, Fibrinogen, and Fibronectin.

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Fusobacterium nucleatum, a gram-negative anaerobic bacterium found in dental plaque, causes periodontal diseases. Smoking is one of the risk factors that can increase periodontal problems and atherosclerosis. Atherosclerosis is initiated by oral bacteria (i.e., *F. nucleatum*) binding to surface proteins of endothelial cells, such as collagen, fibrinogen, and fibronectin. The main objective for this study was to test the binding of *F. nucleatum* to collagen, fibrinogen, and fibronectin under the effect of different concentrations of nicotine. *F. nucleatum* was grown overnight in brain-heart infusion (BHI) supplemented with yeast extract and 5% vitamin-K/hemin. Biofilm was grown for 48 hours in 0, 0.25, 0.5, 1, and 2 mg/mL of nicotine. Then, the biofilm cells were labeled with biotin 3-sulfo-N-hydroxy-succinimide ester sodium salt and fixed with 10% formaldehyde. A binding assay was conducted by coating a high-binding 96-well microtiter plate with 1 µg/mL of collagen, fibrinogen, or fibronectin. The plate was incubated overnight and blocked with 1% Bovine Serum Albumin (BSA), followed by the biotinylated and nicotine-treated *F. nucleatum* cells. ExtrAvidin-Peroxidase and OPD Peroxidase Substrate was used to visualize the binding. Optical density (OD) was measured with a spectrophotometer at 490 nm. Collagen, fibrinogen, and fibronectin binding assays demonstrated significantly higher absorbance with 2 mg/mL nicotine-treated *F. nucleatum* cells compared to untreated cells. The results indicated that an increase in nicotine concentration leads to an increase in *F. nucleatum* binding to collagen, fibrinogen, and fibronectin. This means that smokers may have an increased risk for atherosclerosis. Supported by Life-Health and Sciences Internship (LHSI).